

AMENDMENTS TO THE CLAIMS

1. – 15. (Cancelled)

16. (Original) A method for separating one or more cells of a second type from a sample, said method comprising the steps of:

(a) introducing into one or more microfluidic channels (i) a sample comprising cells of at least a first and second type and (ii) a solution that preferentially lyses cells of the first type, to cause greater lysis of cells of the first type compared to cells of the second type;

(b) contacting the product of step (a) with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said second type of cell;

(c) collecting cells bound to said obstacles, thereby producing a cell population enriched in said second type of cell;

(d) arraying said cell population enriched in said second type of cell;

(e) identifying one or more cells of said second type in said population enriched in said second type of cell; and

(f) collecting said one or more cells of said second type, thereby separating said one or more cells of said second type from said sample.

17. (Original) A method for separating one or more cells of a second type from a sample, said method comprising the steps of:

(a) introducing into one or more microfluidic channels (i) a sample comprising ~~cells of at least a first, second, and third type~~ and (ii) ~~a solution that preferentially lyses~~ cells of the first type, to cause greater lysis of cells of the first type compared to cells of the second type;

(b) contacting the product of step (a) with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said third type of cell compared to said second type of cell;

(c) collecting cells not bound to said obstacles, thereby producing a cell population enriched in said second type of cell;

(d) arraying said cell population enriched in said second type of cell;

(e) identifying one or more cells of said second type in said cell population enriched in said second type of cell; and

(f) collecting said one or more cells of said second type, thereby separating said one or more cells of said second type from said sample.

18. (Original) A method for producing a population of cells enriched in a second type of cell, said method comprising the steps of:

(a) introducing into one or more microfluidic channels (i) a sample comprising cells of at least a first and second type and (ii) a solution that preferentially lyses cells of the first type, to cause greater lysis of cells of the first type compared to cells of the second type;

(b) contacting the product of step (a) with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said second type of cell; and

(c) collecting cells bound to said obstacles, thereby producing said population of cells enriched in said second type of cell.

19. (Original) A method for producing a population of cells enriched in a second type of cell, said method comprising the steps of:

(a) introducing into one or more microfluidic channels (i) a sample comprising cells of at least a first, second, and third type and (ii) a solution that preferentially lyses

cells of the first type, to cause greater lysis of cells of the first type compared to cells of the second type;

(b) contacting the product of step (a) with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said third type of cell compared to said second type of cell; and

(c) collecting cells not bound to said obstacles, thereby producing said population of cells enriched in said second type of cell.

20. (Cancelled)

21. (Original) A method for separating one or more cells of a second type from a sample, said method comprising the steps of:

(a) contacting a sample comprising cells of at least a first and second type with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said first type of cell compared to said second type of cell;

(b) collecting cells not bound to said obstacles, thereby producing a depleted cell population;

(c) arraying said depleted cell population;

(d) identifying said one or more cells of said second type in said depleted population; and

(e) collecting said one or more cells of said second type, thereby separating said one or more cells of said second type from said sample.

~~22. (Original) A method for separating one or more cells of a second type from a sample, said method comprising the steps of:~~

(a) contacting a sample comprising cells of at least a first and second type with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said second type of cell compared to said first type of cell;

(b) collecting cells bound to said obstacles, thereby producing a depleted cell population;

(c) arraying said depleted cell population;

(d) identifying said one or more cells of said second type in said depleted population; and

(e) collecting said one or more cells of said second type, thereby separating said one or more cells of said second type from said sample.

23. (Original) The method of any one of claims 16-22, wherein said second type of cell is a fetal red blood cell.

24. (Original) The method of any one of claims 16-20, wherein said solution in step (a) comprises NaHCO_3 and acetazolamide.

25. (Original) The method of any one of claims 16-20, further comprising the step, after step (a), of diluting the product of step (a) with a diluent in said one or more microfluidic channels.

26. (Original) The method of any one of claims 16-19, 21, and 22, wherein each of said obstacles is coated with a binding moiety.

27. (Previously Presented) The method of claim 26, wherein said binding moiety comprises an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, or a combination thereof.

28. (Original) The method of any one of claims 16, 17, and 20-22, wherein at least 75%, 80%, 90%, 95%, 98%, or 99% of said one or more cells of said second type in said sample are arrayed in said arraying device.

29. The method of any one of claims 18-19, wherein at least 75%, 80%, 90%, 95%, 98%, or 99% of cells of said second type in said sample are collected.

30. – 43. (Cancelled)

44. (Currently Amended) A method of producing a cell population ~~depleted or~~ enriched in a ~~second~~ first type of cell, said method comprising the steps of:

~~(a) contacting~~ subjecting a blood sample comprising cells of at least a first and second type with to (i) separation comprising contact with a microfluidic channel comprising obstacles so that adult, enucleated red blood cells and cells smaller than adult, enucleated red blood cells are directed in one direction and cells larger than adult, enucleated red blood cells are directed in a second direction and (ii) separation comprising contact with a microfluidic device comprising obstacles that preferentially bind said first type of cell, wherein each of steps (i) and (ii) produce a sample enriched in the first type of cell compared to said second type of cell; and

~~(b) collecting cells bound to said obstacles or collecting cells not bound to said obstacles, thereby producing a depleted or~~ an enriched cell population.

45. (Original) The method of claim 44, wherein said obstacles are coated with a binding moiety that binds to the surface of said first type of cell.

46. (Original) The method of claim 44, wherein said first type of cell is a fetal red blood cell.

47. (Cancelled)

48. (Currently amended) The method of claim 44, wherein at least 60% of cells of said first type in said sample are bound to said obstacles of step (ii).

49. (Currently amended) The method of claim 44, wherein at least 70% of cells of said second type in said sample are not bound to said obstacles of step (ii).

50. (Currently amended) The method of claim 44, wherein said obstacles of step (ii) are ordered in a two-dimensional array.

51. (Previously presented) A microfluidic device for producing a cell population enriched or depleted in a second type of cell comprising obstacles disposed in a microfluidic channel, each of which comprises a fetal-cell specific, epithelial-cell specific, tumor-cell specific, stem-cell specific, bacteria specific, protozoan specific, or fungal specific antibody, wherein, when a sample comprising first and second types of cells is introduced into said device, said first or second type of cell preferentially binds to said obstacles.

52. (Original) The microfluidic device of claim 51, wherein said obstacles are ordered in a two-dimensional array.

53. (Original) The microfluidic device of claim 51, wherein each of said obstacles is between 50 and 500 μm high.

54. (Original) The microfluidic device of claim 51, wherein each of said obstacles has a dimension orthogonal to height that ranges between 5 and 500 μm .

55. (Original) The microfluidic device of claim 51, wherein each of said obstacles is disposed at least 10 to 1000 μm from any other obstacle.

56. (Previously presented) The microfluidic device of claim 51, wherein said antibody is an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, or a combination thereof.

57. (Previously presented) A microfluidic device for producing a cell population enriched or depleted in a second type of cell comprising:

(a) a first region of obstacles disposed in a microfluidic channel defining a fluid flow path, wherein said obstacles in said first region preferentially bind a first type of cell compared to a second type of cell; and

(b) a second region of obstacles disposed in said microfluidic channel, wherein said obstacles in said second region preferentially bind a third type of cell compared to a fourth type of cell,

provided that said first and third types of cells are not the same.

58. (Original) The microfluidic device of claim 57, wherein said second and said fourth types of cells are the same.

59. (Original) The microfluidic device of claim 58, wherein said second and said third types of cells are the same.

60. (Original) The microfluidic device of claim 57, wherein said first region and said second region are arranged in series with regard to fluid flow in said microfluidic channel.

61. (Original) The microfluidic device of claim 57, wherein said first region and said second region are arranged in parallel with regard to fluid flow in said microfluidic channel.

62. (Original) The microfluidic device of claim 61, wherein said obstacles in said first region are interspersed among said obstacles in said second region.

63. (Previously presented) A microfluidic device for producing a cell population enriched or depleted in a second type of cell comprising:

(a) a first region of obstacles disposed in a microfluidic channel defining a fluid flow path, wherein said obstacles in said first region preferentially bind a first type of cell compared to a second type of cell, wherein said obstacles are arranged in at least two substantially parallel rows and in at least two substantially parallel columns; and

(b) a second region of obstacles disposed in said microfluidic channel, wherein said obstacles in said second region preferentially bind a third type of cell compared to a fourth type of cell, wherein said obstacles are arranged in at least two substantially parallel rows and in at least two substantially parallel columns,

wherein the first and second regions are disposed adjacent one another in a microfluidic channel, and wherein the rows in the second region are displaced relative to the rows in the first region by a distance of less than the distance between the rows in the first region.

64. (Original) The microfluidic device of 63, wherein the obstacles in the first region are coated with an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, or a combination thereof.

65. (Original) The microfluidic device of 63, wherein the obstacles in the second region are coated with an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, or a combination thereof.

66. (Original) The microfluidic device of claim 63, wherein the ratio of the distance between the rows in the first region to the distance between the columns in the first region is about $\sqrt{3}$.

67. (Original) The microfluidic device of claim 63, wherein the ratio of the distance between the rows in the second region to the distance between the columns in the second region is about $\sqrt{3}$.

Group VI

68. (Previously Presented) The microfluidic device of claim 51, wherein said binding moiety is a fetal specific binding moiety.

69. (Previously Presented) The microfluidic device of claim 51, wherein said binding moiety comprises an antibody, polypeptide, nucleic acid, synthetic polymer, or carbohydrate.

70. (Currently amended) A method for separating one or more cells of a second type from a sample, said method comprising the steps of:

(a) contacting said sample with a channel comprising obstacles so that adult, enucleated red blood cells and cells smaller than adult, enucleated red blood cells are directed in one direction and cells larger than adult, enucleated red blood cells are directed in a second direction to produce a first enriched sample;

(b) contacting said first enriched sample with a device comprising obstacles comprising a binding moiety that preferentially binds cells of a first type compared to cells of said second type; and

~~(b)~~ (c) releasing cells bound to said obstacles, thereby separating said cells from said sample.

71. (Previously presented) The method of claim 70, wherein said releasing in step (c) comprises applying a shear force or lysing said bound cells.

72. (Currently amended) The method of claim 70, further comprising arraying said cells after step ~~(b)~~ (c).

73. (Currently amended) The method of claim 70, further comprising analyzing the cellular contents of said cells after step ~~(b)~~ (c).

74. (Previously presented) The method of claim 73, wherein said analyzing comprises FISH.

75. (Previously presented) The method of claim 73, wherein said analyzing comprises nucleic acid analysis.

76. (Previously presented) The method of claim 70, wherein said first type comprises fetal cells, epithelial cells, tumor cells, stem cells, bacteria, protozoa, or fungi.

77. (Currently amended) The method of claim 70, identifying one or more cells of said first type after step ~~(b)~~ (c).

78. (Previously presented) The method of claim 70, wherein said binding moiety comprises an antibody.

79. (Previously presented) The method of claim 78, wherein said antibody is a fetal-cell specific, epithelial-cell specific, tumor-cell specific, stem-cell specific, bacteria specific, protozoan specific, or fungal specific antibody.

80. (Previously presented) The method of claim 70, wherein said second type comprises white blood cells or red blood cells.

81. (Currently amended) A method for identifying one or more cells of a second type in a sample, said method comprising the steps of:

(a) contacting said sample with a channel comprising obstacles so that adult, enucleated red blood cells and cells smaller than adult, enucleated red blood cells are directed in one direction and cells larger than adult, enucleated red blood cells are directed in a second direction to produce a first enriched sample;

(b) contacting said first enriched sample with a device comprising obstacles comprising a binding moiety, wherein said binding moiety preferentially binds cells of said second type compared to cells of a first type; and

~~(b) (c) staining cells bound to said obstacles to identify cells of said second type,~~
wherein said second type is selected from the group consisting of fetal cells, epithelial cells, tumor cells, stem cells, bacteria, protozoa, and fungi.

82. (Currently amended) The method of claim 81, further comprising analyzing the cellular contents of said cells during or after step ~~(b)~~ (c).

83. (Previously presented) The method of claim 82, wherein said analyzing comprises nucleic acid analysis.

84. (Previously presented) The method of claim 81, wherein said staining comprises FISH.

85. (Previously presented) The method of claim 81, wherein said second type comprises fetal cells, epithelial cells, or tumor cells.

86. (Currently amended) The method of claim 81, identifying one or more cells of said second type during or after step ~~(b)~~ (c).

87. (Previously presented) The method of claim 81, wherein said binding moiety comprises an antibody.

88. (Previously presented) The method of claim 87, wherein said antibody is a fetal-cell specific, epithelial-cell specific, tumor-cell specific, stem-cell specific, bacteria specific, protozoan specific, or fungal specific antibody.

89. (Previously presented) The method of claim 81, wherein said first type comprises white blood cells or red blood cells.

90. (Previously presented) A kit comprising (i) a device of claim 51, and (ii) a lysis reagent capable of lysing said first or second type of cell bound to said obstacles.

91. (Previously presented) A kit comprising (i) a device of claim 51, and (ii) a stain for cells of said first or second type of cell bound to said obstacles.

92. (Previously presented) A kit comprising (i) a device of claim 51, and (ii) a reagent for nucleic acid analysis of cells of said first or second type of cell bound to said obstacles.

93. (Previously presented) A method of manufacturing a device for producing a cell population enriched or depleted in a second type of cell, said method comprising:

- (a) providing a substrate;
- (b) creating a plurality of obstacles on said substrate;
- (c) attaching a fetal-cell specific, epithelial-cell specific, tumor-cell specific, stem-cell specific, bacteria specific, protozoan specific, or fungal specific antibody to said plurality of obstacles; and
- (d) enclosing said plurality of obstacles to generate a fluidic channel.

94. (Previously presented) The method of claim 93, wherein the substrate is selected from a silicon-based substrate, a metal-based substrate, a silicon-on insulator substrate, a glass substrate or a polymeric-based substrate.

95. (Previously presented) The method of claim 93, wherein the creating step comprises the use of lithography, micromachining, casting, molding, embossing, wet chemical etch, dry chemical etch, milling, diamond cutting, electroforming, LIGA, or any combination thereof.

96. (Previously presented) The method of claim 93, wherein the pattern of obstacles is a two dimensional array.

97. (Previously presented) The method of claim 96, wherein the two dimensional array pattern comprises a plurality of rows wherein every subsequent row is shifted relative to a previous row.

98. (Previously presented) The method of claim 93, wherein the antibody is selected from the group consisting of anti-CD71, anti-CD36, anti-GPA, and anti-CD45.

99. (Previously presented) A fetal cell enrichment device comprising one or more binding moiety that selectively binds fetal cells or non-fetal cells and a matrix of one or more hydrogel polymers.

100. (Previously presented) A method for separating fetal cells from a sample comprising fetal and non-fetal cells comprising: flowing said sample through a matrix comprising a hydrogel polymer -containing a binding moiety that selectively binds fetal red blood cells or a non-fetal cell, thereby separating said fetal cells from a subset of said non-fetal cells.

101. (Previously presented) A tumor cell enrichment device comprising one or more binding moiety that selectively binds tumor cells or non-tumor cells and a matrix of one or more hydrogel polymers.

102. (Previously presented) A method for separating tumor cells from a sample comprising tumor and non-tumor cells comprising: flowing said sample through a matrix comprising a hydrogel polymer -containing a binding moiety that selectively binds tumor

cells or a non-tumor cell, thereby separating said tumor cells from a subset of said non-tumor cells.

103. (Previously presented) A system comprising:

one or more size-based separation regions, wherein each of said size based separation regions comprise a plurality of obstacles that direct a first analyte of a fluid sample in a first direction and a second analyte of said fluid sample in a second direction, wherein said first analyte and second analyte have a different size; and

one or more capture regions that selectively captures either said first analyte, second analyte or a third analyte.

104. (Previously presented) A method for size-based separation of a first analyte from a second analyte of a different size in a fluid sample comprising:

applying said sample to one or more size-based separation regions and one or more capture regions, wherein each said size-based separation region comprises a plurality of obstacles which direct said first analyte in a first direction and said second analyte in a second direction and each said capture region selectively captures said first, second, or a third analyte.

105. (Previously presented) A device for producing a cell population enriched in a first cell type from a blood sample comprising a first inlet in communication with a channel comprising two rows of obstacles, wherein said two rows of obstacles direct said first cell type towards a first outlet in a first direction and a second cell type towards a second outlet in a second direction.

106. (Previously presented) A device for producing a cell population enriched in fetal red blood cells from a maternal blood sample comprising a first inlet in

communication with a channel comprising a first plurality of gaps, wherein said first plurality of gaps directs said one or more fetal red blood cells towards a first outlet in a first direction and one or more maternal red blood cells towards a second outlet in a second direction.

107. (Previously presented) A device for producing a cell population enriched in epithelial cells from a blood sample comprising a first inlet in communication with a channel comprising a first plurality of gaps, wherein said first plurality of gaps directs said one or more epithelial cells towards a first outlet in a first direction and one or more non-epithelial cells towards a second outlet in a second direction.

108. (Previously presented) A method for producing a cell population enriched in a first cell type from a blood sample, said method comprising: applying said blood sample to a device having a first inlet in communication with a channel comprising a plurality of obstacles, wherein, when said blood sample flows through said channel, said plurality of obstacles direct said first cell type towards a first outlet in a first direction and a second cell type in said blood sample towards a second outlet in a second direction, thereby producing said cell population enriched in said first cell type.

109. (Previously presented) A method for producing a cell population enriched in fetal red blood cells from a maternal blood sample, said method comprising: applying said maternal blood sample to a device having a first inlet in communication with a channel comprising a plurality of obstacles, wherein, when said maternal blood sample flows through said channel, said plurality of obstacles direct said fetal red blood cells towards a first outlet in a first direction and one or more non-fetal red blood cells in said maternal blood sample towards a second outlet in a second direction thereby producing said cell population enriched in fetal red blood cells.

110. (Previously presented) A method for producing a cell population enriched in epithelial cells from a blood sample, said method comprising: applying said blood sample to a device having a first inlet in communication with a channel comprising a plurality of obstacles, wherein, when said blood sample flows through said channel, said plurality of obstacles direct said epithelial cells towards a first outlet in a first direction and one or more non-epithelial cells in said blood sample towards a second outlet in a second direction, thereby producing said cell population enriched in epithelial cells.

111. (Previously presented) A method for producing a cell population enriched in tumor cells from a blood sample, said method comprising: applying said blood sample to a device having a first inlet in communication with a channel comprising a plurality of obstacles, wherein, when said blood sample flows through said channel, said plurality of obstacles direct said tumor cells towards a first outlet in a first direction and one or more non-tumor cells in said blood sample towards a second outlet in a second direction, thereby producing said cell population enriched in tumor cells.

112. (Previously presented) A device comprising a first array of obstacles disposed to selectively enrich, based on size, shape, or deformability, a first cell type having a concentration of up to 50×10^{-3} cells/ μl in a sample by a factor of at least 100,000.

113. (Previously presented) A device comprising a first array of obstacles that depletes one or more non-fetal cells from a maternal blood sample and recovers at least 90% of fetal cells present in said maternal blood sample.

114. (Previously presented) A device comprising a first array of obstacles that depletes one or more non-tumor cells from a blood sample and retains at least 90% of tumor cells in said blood sample.

115. (Previously presented) A method for producing a cell population enriched in a first cell type which is present in a sample at a concentration of up to 50×10^{-3} cells/ μ l comprising:

applying said sample to a device comprising a first array of obstacles to produce said cell population which is enriched in said first cell type by a factor of at least 100,000.

116. (Previously presented) A method for manufacturing a device for cell sorting comprising:

providing a substrate; and

creating a pattern of obstacles on said substrate, wherein said obstacles allow passage of cells based on their size, shape or deformability,

wherein the substrate comprises at least two outlets for collecting the sorted cells.

117. (New) The method of claim 44, wherein the preferential binding in step (ii) is reversible.

118. (New) The method of claim 117, wherein said reversible preferential binding is actuated by a field.